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BIOTIN

Since the concept of a vitamin was first introduced thirty years ago, tremendous progress has been made in this field. In that period scientists have been able to isolate and to synthesize a great many vitamin factors and to indicate the possible existence of still others, so that at present the list of known and postulated vitamins totals close to fifty. The function of many of the vitamins has been empirically recognized; nutritional deficiency diseases have in many cases been attributed to the lack of specific vitamins and likewise cured by their administration; the relationships between vitamins and other factors such as enzymes and hormones has become apparent; and the first glimmers of light with respect to the complex mechanisms involved in the biological action of the vitamins are becoming clear.

The book is not closed, however, with respect to the story of the vitamins. One of the most fascinating chapters concerns the recent recognition of a new and extremely potent vitamin, *biotin*. Biotin has been found essential for every type of living organism and according to experimental indications is intimately involved in the vital processes of cell growth. Biotin was originally considered only as a growth factor for yeast and was later discovered to be identical with a respiration enzyme for certain micro-organisms. Only recently was it recognized that these two factors were one and the same with the animal nutrition factor, vitamin H—and that all three substances were merely different biological manifestations of the same compound.

Biotin is very widespread in its occurrence, being found in most foods. It is especially prevalent in yeast, liver, molasses and milk. Biotin was first isolated in minute quantities from egg yolk and later from liver, but more recently it has been possible to obtain pure crystalline biotin on a larger scale from milk. Furthermore it is so highly potent that the merest traces suffice for ordinary physiological needs. It is ten times more active than any of the known B vitamins and functions as an effective yeast growth factor in a concentration as little as one part in 400 billion. For this reason a biotin deficiency disease had never been observed until the use of an unusual experimental diet indirectly brought it to light. The unusual feature of this diet was a large amount of uncooked egg-white used to supply the entire requirement of protein. Strangely enough, animals on the diet containing this thoroughly nutritious

form of protein developed a nutritional disorder designated as "egg-white injury" which had never before been observed. It was found that certain foods were able to counteract the effect of the raw egg-white. Investigation into the protective nature of these foods led to the discovery of the so-called "anti-egg-white injury factor", which is now known as vitamin H or biotin. After many years of patient investigation it was discovered that the harmful effects of raw egg white were caused by some constituent which had the power of combining with any biotin present in the diet and thus rendering it unavailable for use by the animal. *Heating or cooking the egg-white destroyed the biotin-binding ingredient and prevented the development of the characteristic injury.* The mysterious ingredient in egg-white was finally isolated and because of its affinity for biotin, named *avidin* or *anti-biotin*. Avidin is the first anti-vitamin to be discovered in the science of nutrition and represents a hitherto unknown mechanism in the production of disease. Because of its anti-vitamin characteristics, avidin brings about a *secondary nutritional deficiency* by rendering dietary biotin unavailable. Thus the presence of excessive amounts of avidin in effect achieves the same result as would a true absence or deficiency of biotin.

The significance of the biotin-avidin complex in human nutrition and in the nutrition of animals, especially poultry, is now being investigated and promises to be of tremendous import. (N.B. This phase of biotin research will be discussed in Part II of this paper next month.)

The Discovery of Biotin

Bios: About forty years ago, a group of Belgian scientists (1) studying the various nutrients essential to keep yeast cells alive and growing, found that yeast required among other things, traces of a certain factor in the culture media without which growth is impossible. They called the unknown factor "bios" meaning "life." As progress was made in the study of the new factor it became apparent that bios was not a single factor but was composed of several compounds or fractions each of which, upon separation, exhibited different biological activity. About thirty-five years later an extremely potent yeast growth factor was isolated from one of the bios fractions by Kögl and Tönnis in Holland (2). They named the new factor "biotin" and in 1936 succeeded in isolating a little over 1 mg. of biotin in crystalline form from 250 kilos of dried egg yolk.

Coenzyme R: The discovery of biotin by Kögl and Tönnis constituted only one chapter in the remarkable story of this vitamin. At the same time, Allison, Hoover and Burk (3) in the United States were engaged in a totally different problem. These workers were seeking another growth factor found to be essential for the growth of the root-nodule bacterium *Rhizobium*. Because the unknown factor which they were seeking exerted a profound effect upon the res-

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piration of Rhizobia, it was designated as a respiration coenzyme. This name was later shortened to "coenzyme R." In 1939, a few years after the isolation of biotin, West and Wilson (4) concluded from their experiments that coenzyme R and biotin were either identical or that each was active as a growth stimulant for either or both yeast and Rhizobia. Further research with the two factors confirmed the supposition that they were identical and coenzyme R officially became biotin.

Vitamin H: Nine years before biotin was isolated from bios by Kögl and Tönnis, and while the work on coenzyme R was still in its early stages, remarkable discoveries were being made by another group of investigators in the field of animal nutrition.

It had been discovered, about 1927, that rats fed uncooked dried egg-white as the sole source of protein in the diet instead of the usual casein, frequently developed characteristic symptoms including dermatitis, nervous disorder, loss of hair, decreased weight, and loss of muscular control. Continuation of the condition eventually led to death. This unusual condition was first observed by Boas (5) who named it "egg-white injury."

It was later found that the disease could be prevented or cured either by cooking the egg-white or by supplying certain foods which seemed to counteract the effect of the raw egg-white. This led Boas to assume that these foods contained a specific factor protecting against the so-called "egg-white injury." She named the unknown substance "protective factor X" or the "anti-egg-white injury factor;" this was later changed to "vitamin H" by György (6) who suggested that the factor was a vitamin.

It was not until several years later that the suspicion arose that the yeast factor, *biotin,* and the Rhizobium factor, *coenzyme R,* might bear some relation to the animal nutrition factor, *vitamin H.* The story of how all these three factors came to be recognized as a single entity, *biotin,* involves a number of remarkable coincidences as well as a great deal of ingenious scientific research.

The Story of Vitamin H

Egg-White Injury: In 1927 when Boas (5) first observed the characteristic syndrome in rats fed uncooked dried egg-white as the only dietary protein, a series of remarkable investigations was initiated. The mystery of how a protein of established nutritional value and wholesomeness could produce toxic symptoms under certain conditions presented a puzzle which required the combined efforts of many scientific investigators over a period of several years to solve. Boas found, after careful investigation of the field, that other workers using the same diet she employed did not report any egg-white injury in their animals. Upon minute consideration of the diets used by herself and by others, she noted that the only difference was in the type of starch used. Boas used wheat starch, whereas the other investigators had been using potato starch.

Following up this clue, experiments were conducted with both types of starch and it was found that when potato starch was supplied the animals failed to develop the egg-white injury, but that when wheat starch was given, they did. As a result of these experiments, Boas suggested that raw egg-white contained a "toxic" factor responsible for the symptoms of egg-white injury. She suggested further that potato starch must contain an unknown factor which protects against egg-white injury and which is not present in wheat starch. From the occurrence and distribution of the "protective factor X," in various foods, Boas believed it might in some way be connected with the members of the vitamin B complex.

Two years after the publication of the paper by Boas describing the disorder produced in laboratory animals by the feeding of uncooked dried egg-white, it was suggested by Findlay and Stern (7) that Swift's disease in children ("pink disease," so called because of the characteristic pinkness of the extremities) was related to egg-white injury in rats. The disease in children was characterized by symptoms corresponding to those of the animal disorder, was not accompanied by fever and was not infectious. They noted further that the disease was restricted to young animals and to children—never afflicting adults. From the nature of the disease, Findlay and Stern suggested a possible connection with the nutritional deficiency diseases then known, such as beri-beri, scurvy, and the like. The fact that suckling animals developed the disease when reared on breast milk from mothers fed the raw egg-white diet was interpreted as further proof that the disease was probably due to a deficiency in the mother's milk resulting from the raw egg-white diet. In cases of human deficiency diseases, similarly, children nursed by mothers whose diets are deficient develop characteristic diseases because of inadequacies inherent in the mother's milk. Findlay and Stern concluded that if egg-white injury in rats was actually a type of deficiency disease, and if the disease was related to "pink disease" in man, then the protective factor described by Boas must be essential in human nutrition.

In the meantime other workers were conducting studies on the nature of the substance in egg white responsible for the "toxic" symptoms. About two years after the report of Findlay and Stern, Parsons (8) found that merely drying egg-white was not responsible for the symptoms. She demonstrated that fresh raw egg-white when used as the sole source of protein could also produce the characteristic egg-white injury. She made the significant observation, however, that *heating* egg-white destroyed its "toxic" effect. As a result of her observations Parsons made a preliminary suggestion that: "the injury appears to involve an interrelationship between a positive toxicity and a *relative* absence of a protective factor."

Parsons subsequently made extensive studies of the egg-white injury factor and of the factor which protected against it. Various foods were tested for the

presence of the protective factor. She found it occurred in cooked beef liver and kidney, pork liver and kidney, dried yeast, egg yolk, wheat embryo, and dried milk (9). Chemical analyses showed that the dermatitis–producing factor in the egg-white could be destroyed by peptic digestion or mild acid treatment; that the activity resided in the protein fraction of the egg-white, but that it was not associated with purified egg albumin (10). Parsons' work was later confirmed by Salmon and Goodman (11). They also indicated, as did Parsons, that the injury was not an anti-vitamin G effect, despite the fact that the symptoms were somewhat similar to the skin lesions resulting from vitamin G deficiency.

After about six years of continued research, the first definite indication of the mechanism involved in egg-white injury appeared when Parsons showed that egg-white is not "toxic" because it lacks an essential factor or contains an anti-enzyme, but because it probably *combines with and thus inactivates in the digestive tract a protective factor in the diet* (12). Accordingly, it was concluded that the "curative" quality of heated or cooked egg-white depended on the destruction of its combining capacity.

A New Vitamin: Supplementing Parsons' initial studies on the nature and occurrence of the factor protecting against egg-white injury, a great deal of research was devoted to elucidating the chemical and physical properties of the unknown factor. A major part of the work in this field was carried on by György at Western Reserve University, with the assistance of Catharine Rose. György was much impressed with the similarity of symptoms in the rat to certain clinical conditions in man. He thought that the symptoms resembled a class of dermatitis in man characterized by seborrhea, and scaly desquamation (as, for example, psoriasis or acne). György also suspected that the so-called egg-white injury was an induced deficiency disease, so that he was led to call the unknown protective factor a vitamin. He named the new vitamin "vitamin H" from the German word Haut (skin), to indicate it was a vitamin the lack of which produced severe skin lesions (6).

Vitamin H and Biotin: From 1931 on, when György first introduced the term "vitamin H" he conducted a vast number of experiments in an attempt to isolate the vitamin and to study its properties. Since the vitamin H preparations with which György and his group were working were only crude concentrates, it was difficult to obtain precise data as to the physical and chemical properties of the compound. At this point, György was joined by du Vigneaud, Melville and Burk at Cornell University, who participated in the chemical aspects of the vitamin H investigations. Through the efforts of the group at Cornell, the vitamin H concentrates were further purified to a potency some 15 to 20 times greater than the original concentrates.

It was a curious coincidence that, during the course of this work, the thought occurred simultaneously to both groups of investigators that vitamin

H might be biotin. This thought arose as a result of Melville's observation that many of the vitamin H concentrates exhibited extremely high biotin activity; it also occurred separately to the Western Reserve group as a result of the many similarities noted between the properties of their vitamin H and of biotin described in the literature. Tests were immediately instituted in du Vigneaud's laboratory to substantiate this important conclusion. Results of preliminary experiments were indeed encouraging and it began to look as though the mystery of the identity of vitamin H was about to be solved. A further coincidence in this work was the fact that Burk, working with du Vigneaud and Melville, was one of the co-discoverers some seven years previously of coenzyme R which had already been shown to be identical with biotin and which now gave every indication of being vitamin H as well.

With the availability of pure crystalline biotin, generously supplied by Kögl, it became possible to confirm preliminary reports (13) that vitamin H and biotin, and of course coenzyme R, were indeed identical (14, 15). This meant that biotin, initially found important for yeast and bacteria, was also an important compound in the vital economy of animals. Thus, *biotin* isolated in 1936 by Kögl and Tönnis, *coenzyme R* discovered in 1933 by Allison, Hoover and Burk, and *vitamin H* postulated by György in 1931, became recognized as a single biological entity in 1940, thirteen years after Boas' first discovered the "protective factor X" which cured the mysterious egg-white injury.

Biotin and Avidin: With the isolation of pure crystalline biotin from egg yolk (2) and from liver (16) and with recognition of its various biological functions in both plant and animal spheres, new impetus was given to the study of the biological action of biotin, or vitamin H. One very important question concerned the manner in which biotin prevented the characteristic symptoms developed in animals on a diet containing large amounts of raw egg white. As Parsons had suggested a few years previously (12) the protective factor (now known to be biotin) was probably rendered unavailable in the digestive tract by combining with some constituent of raw egg-white, and the "curative" properties of cooked egg-white were dependent upon the destruction by heat of its combining capacity.

In 1940, Eakin, McKinley, and Williams at the University of Texas (17) observed that the tissues of chicks kept on a diet causing egg-white injury were extremely deficient in biotin despite the abundance of this vitamin in the diet. This led them to conclude, like Parsons, that the injury caused by egg-white must be due to some action of raw egg-white in making biotin unavailable. To test this supposition, it was decided to demonstrate if possible the *in vitro* inactivation of biotin by egg-white (18). Accordingly, an amount of egg-white was introduced into two sterile biotin solutions, one of which was heat-treated or cooked. The two solutions were then tested for biotin according to the standard method of Snell, Eakin, and Williams (19). As suspected, biotin in the solution containing unheated egg-white was inactivated, whereas it was readily

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recovered from the cooked egg-white solution. This confirmed the belief that some constituent of uncooked egg-white was capable of inactivating biotin, thus producing "toxic" symptoms by bringing about a deficiency of biotin. Further studies confirmed a similar action *in vivo*. This led to the recognition of egg-white "toxicity" as a *secondary deficiency disease* caused by the biological unavailability of the vitamin, biotin.

Following this new light thrown on the mechanism of egg-white injury, efforts were directed toward isolating the constituent of egg-white responsible for inactivating biotin. In preliminary purifications of the active fraction of egg-white it was noted that the biotin-binding constituent exhibited albumin-like properties. Accordingly, it was named *avidalbumin* (hungry albumin) by the Texas group. This was later shortened to *avidin*. After numerous experiments *avidin*, the first recognized "anti-vitamin," was finally isolated in crystalline form and its specificity in combining with biotin confirmed (20). The existance of a biotin-binding constituent in raw egg-white was also confirmed by Woolley and Longsworth (21) who named it "anti-biotin." The mechanism of egg-white injury, now finally explained, represents a hitherto unknown principle in the production of a nutritional disease; namely *a secondary deficiency disease brought about despite the abundant presence of a vitamin in the diet through the action of another food constituent in rendering the necessary vitamin physiologically unavailable.*

The significance of biotin and avidin in the nutrition and physiology of animals and of man will be described in part II of this article in the March issue of *Borden's Review of Nutrition Research.*

For further information or additional copies address:
RUTH WOODS, *Editor*, Borden's Review of Nutrition Research
350 Madison Avenue, New York, N. Y.

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